

Amendments to the Claims

1. (Previously Presented) A method of producing male or female sterile plants comprising the steps of:
 - a) transforming plant material with a polynucleotide which encodes at least one enzyme which reacts with a non-phytotoxic substance to produce a phytotoxic one, wherein the enzyme is expressed preferentially in either male or female reproductive structures and characterized in that the enzyme is a mutant D-amino acid oxidase, derived from *Rhodotorula gracilis*, said oxidase comprising a lysine at position 58 rather than a phenylalanine in the wild type sequence, and wherein the non-phytotoxic substance is characterized as a D-alpha amino acid, or a peptide derivative of a non-protein D-alpha amino acid;
 - b) regenerating the thus transformed material into a plant;
 - c) applying the said non-phytotoxic substance to the plant up to the time of male or female gamete formation and/or maturation, so that the non-phytotoxic substance provides for the production of a phytotoxic one which selectively prevents the formation of or otherwise renders the said gametes non-functional; and
 - d) selecting for the plant comprising either male or female sterility.
2. (Original) A method according to claim 1, wherein the said non-phytotoxic substance is applied in mixture along with at least one further substance which is selected from the group consisting of safeners, gametocides, glutathione-S-transferase inducers, cytochrome P450 inducers, herbicides, fertilizers, nematocides, synergists, insecticides, fungicides, hormones, plant-growth regulators and cytochrome P450 inhibitors.
3. (Previously Presented) A method according to claim 1, wherein the non-phytotoxic substance is applied foliarly and is a phloem mobile and metabolically stable oxidisable substrate of the enzyme, wherein the enzyme provides the phytotoxic product, as a direct or indirect one from the non-phytotoxic substance.
4. (Previously Presented) A method according to the claim 3, wherein the phytotoxic product is an indirect one produced in the form of peroxide.
5. (Previously Presented) A method according to claim 3, wherein the non-phytotoxic substance is D-aspartate or D-glutamate and the enzyme oxidises the said amino acid to a 2-keto acid with concomitant reduction of oxygen to a peroxide anion.
6. (Previously Presented) A method according to claim 1 wherein the enzyme comprises a substitution at position 213 when compared to the wild type sequence.
7. (Previously Presented) A method according to claim 6, wherein the enzyme has at position 213 an amino acid selected from the group consisting of His, Ser, Thr, Cys, Gin, Gly, Asn and Ala.

8. (Previously Presented) A method according to claim 7 wherein the amino acid at position 213 is selected from the group consisting of Ser and Thr.
9. (Previously Presented) A method according to claim 3, wherein the enzyme is targeted to other than the peroxisome.
10. (Previously Presented) A method according to claim 1, wherein the non-phytotoxic substance is one or more substances selected from the group consisting of the D-enantiomer of phosphinothricin and a D- enantiomer of bialaphos.
11. (Previously Presented) A method according to claim 1, wherein the non-phytotoxic substance is comprised within a mixture, which contains a phytotoxic substance
12. (Previously Presented) A method according to claim 11 wherein the enzyme is a mutant D-amino acid oxidase derived from *Rhodotorula gracilis* which oxidase comprises substitutions in at least one position selected from the group consisting of 213, 238, or 223 when compared to the wild type sequence.
13. (Previously Presented) A method according to claim 12, wherein the oxidase derived from *Rhodotorula gracilis* has at position 213 an amino acid selected from the group consisting of: His, Ser, Thr, Cys, Gln, Gly, Asn and Ala, and at position 223 an amino acid selected from the group consisting of: His, Ser, Thr, Cys, Gln, Gly, Asn and Ala.
14. (Previously Presented) A method according to claim 13 where the amino acid at position 213 is selected from the group comprising Ser and Thr.
15. (Previously Presented) A method according to claim 10, wherein the mixture comprises both D- and L- phosphinothricin and the plant material expresses a PAT gene substantially in green tissues.
16. (Previously Presented) A mutant D-amino acid oxidase derived from *Rhodotorula gracilis*, capable of oxidising phosphinothricin, which comprises a lysine at position 58 rather than a phenylalanine in the wild type sequence.
17. (Previously Presented) An oxidase according to claim 16, further comprising amino acid substitutions in at least one position selected from the group consisting of 213, 223, and 238.
18. (Previously Presented) A method of producing male or female sterile plants comprising the steps of:
 - a) transforming plant material with a polynucleotide which encodes at least one enzymewhich reacts with a non-phytotoxic substance to produce a phytotoxic one, wherein the enzyme is expressed preferentially in either male or female reproductive structures and characterized in that the enzyme is a mutant D-amino acid oxidase, derived from *Rhodotorula gracilis*, said oxidase comprising a lysine at position 58 rather than a phenylalanine in the wild type sequence and a serine at

position 213 rather than a methionine in the wild type sequence, and wherein the non-phytotoxic substance is characterized as a D-alpha amino acid, or a peptide derivative of a non-protein D-alpha amino acid;

- b) regenerating the thus transformed material into a plant;
 - c) applying the said non-phytotoxic substance to the plant up to the time of male or female gamete formation and/or maturation, so that the non-phytotoxic substance provides for the production of a phytotoxic one which selectively prevents the formation of or otherwise renders the said gametes non-functional; and
 - d) selecting for the plant comprising either male or female sterility.
19. (Previously Presented) The method according to claim 3, wherein the phytotoxic product is an indirect one produced in the form of a super oxide anion.
20. (Previously Presented) The method according to claim 1 wherein the enzyme comprises a substitution at position 223 when compared to the wild type sequence.
21. (Previously Presented) The method according to claim 1 wherein the enzyme comprises a substitution at position 238 when compared to the wild type sequence.
22. (Previously Presented) The method according to claim 7, wherein the enzyme has at position 238 an amino acid selected from the group consisting of His, Ser, Thr, Cys, Asn, Gln, Gly and Ala.
23. (Previously Presented) The method according to claim 7, wherein the enzyme has at position 223 an amino acid selected from the group consisting of His, Ser, Thr, Cys, Ala, Gly, Gln and Asn.
24. (Previously Presented) The method according to claim 16 wherein the enzyme is a mutant D-amino acid oxidase derived from *Rhodotorula gracilis* which oxidase is a D-aspartate oxidase.
25. (Previously Presented) The method according to claim 12, wherein the oxidase derived from *Rhodotorula* has position 238 an amino acid selected from the group consisting of His, Ser, Thr, Cys, Gln, Gly, Asn and Ala, and at position 223 an amino acid selected from the group consisting of: His, Ser, Thr, Cys, Gln, Gly, Asn and Ala.
26. (Previously Presented) The method according to claim 15, wherein the mixture comprises both D- and L- phosphinothricin and the plant material expresses a PAT gene substantially in floral tissue which produce gametes being other than those that are rendered non-functional.
27. (New) A method of producing male or female sterile plants comprising the steps of:
- a) transforming plant material with a polynucleotide which encodes at least one enzyme which reacts with a non-phytotoxic substance to produce a phytotoxic one,

wherein the enzyme is expressed preferentially in either male or female reproductive structures and characterized in that the enzyme is a mutant D-amino acid oxidase having a lysine at the position in its sequence corresponding to residue 58 of the wild-type D-amino acid oxidase sequence from *Rhodotorula gracilis*, and wherein the non-phytotoxic substance is characterized as a D-alpha amino acid, or a peptide derivative of a non-protein D-alpha amino acid;

- b) regenerating the thus transformed material into a plant;
- c) applying the said non-phytotoxic substance to the plant up to the time of male or female gamete formation and/or maturation, so that the non-phytotoxic substance provides for the production of a phytotoxic one which selectively prevents the formation of or otherwise renders the said gametes non-functional; and
- d) selecting for the plant comprising either male or female sterility.